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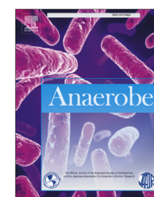

Research paper

Performance of mass spectrometric identification of clinical *Prevotella* species using the VITEK MS system: A prospective multi-center study☆

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ABSTRACT

Prevotella species, members of the human microbiota, can cause opportunistic infections. Rapid and accurate identification of *Prevotella* isolates plays a critical role in successful treatment, especially since the antibiotic susceptibility profile differs between species. Studies, mostly carried out using the Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) Biotyper system, showed that MALDI-TOF MS is an accurate, rapid and satisfactory method for the identification of clinically important anaerobes. In this multi-center study, we assessed the performance of the MALDI-TOF MS VITEK MS system for the identification of clinical *Prevotella* isolates. A total of 508 *Prevotella* isolates, representing 19 different species, collected from 11 European countries, Kuwait and Turkey between January 2014 and April 2016, were identified using VITEK MS (v3.0). The reliability of the identification was assessed by 16S rRNA gene sequencing. Using VITEK MS, 422 (83.1%) of the 508 isolates were identified on the species level, 459 (90.4%) on the genus level. A total of 49 (9.6%) isolates were not identified correctly. 16S rRNA gene sequencing results showed that this was partly due to the fact that several species were not represented in the database. However, some species that were represented in the database were also not identified. Five *Prevotella* strains were misidentified at the genus level, 2 of these strains belonged to a species not represented in the database. In general, the VITEK MS offers a reliable and rapid identification of *Prevotella* species, however the databases needs to be expanded.
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1. Introduction

Prevotella species are part of the oral, upper respiratory, intestinal and female genital tract commensal microbiota. However, they can also cause opportunistic infections, including specific oral infections [1]. As for other anaerobic genera, the phenotypic identification of *Prevotella* species is cumbersome and not always accurate [2]. Nucleic acid sequencing methods are applied in order to reach reliable identifications. However, these methods are expensive, technically complex and too labor intensive for routine

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identification of clinical isolates [3].

The introduction of Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) had a revolutionary impact on the identification of anaerobic bacteria. It was shown to be faster, cheaper and more reliable than traditional methods [4–8]. Currently, two MALDI-TOF MS systems are commercially available for routine use: the MALDI-TOF MS Biotyper (Bruker Daltonics Inc., Germany) and VITEK MS (bioMérieux Inc., France). Although the methodology is similar, there are definitive differences between the two systems in terms of the database composition and the application of software packages for data analysis [9].

Recently, taxonomic changes have been made to the *Prevotella* genus and new species have been added, extended to now include 50 validated species (http://www.bacterio.net/prevotella.html) [10,11]. The antibiotic susceptibility profile differs between the species [1,12]. Significant interspecies differences in antimicrobial resistance were observed for penicillin, clindamycin metronidazole and tetracycline. It has been reported that *P. bivia*, *Prevotella* species most frequently isolated from clinical specimens, was the most resistant species to these antimicrobial agents, and also included multidrug resistant strains [13]. Therefore, it is important that clinical isolates are correctly identified at the species level, in order to make successful treatment possible.

Studies, assessing the performance of MALDI-TOF MS for the identification of different *Prevotella* species, were mostly performed using the MALDI-TOF MS Biotyper system [14,15]. The aim of this multi-center study was to evaluate the performance of the VITEK MS system for the identification of clinically relevant *Prevotella* species. MALDI-TOF MS identification results were compared with 16S rRNA gene sequencing, which is considered the reference method.

2. Methods

2.1. Bacterial isolates

A total of 508 non-duplicate *Prevotella* strains isolated from different clinical samples of non-hospitalized or hospitalized patients were collected from 11 European countries [Austria (n = 29), Belgium (n = 45), Croatia (n = 33), Denmark (n = 45), France (n = 45), Germany (n = 34), Great Britain (n = 45), Greece (n = 18), Hungary (n = 47), Netherlands (n = 45) and Slovenia (n = 29)], Kuwait (n = 14) and Turkey (n = 79), between January 2014 and April 2016. The strains were mainly isolated from abscesses (37.4%) and wounds (18.1%). Other major categories of clinical samples were from intraoral infections (17.4%), soft tissue (5.6%), and bone biopsies (2.1%), non-blood sterile body fluids (6.1%), and blood (1.6%). Each laboratory identified its own isolates at the genus or species level by using conventional and/or modern diagnostic tests including MALDI-TOF MS (Biotyper MS, Bruker, Germany (9 countries) or VITEK MS, bioMerieux, France (3 countries)). The organisms were sent to Turkey, in anaerobic transport medium (Anaerobe systems, Morgan Hill, USA). Upon arrival the strains were immediately cultured on Brucella Blood Agar (BBA) (Difco, USA), supplemented with hemin and vitamin K. Viable strains were stored at −80 °C in 10% skimmed milk until use. The study was carried out at the Department of Clinical Microbiology, School of Medicine, Marmara University, Istanbul, Turkey.

2.2. Identification by VITEK MS

All isolates were grown on BBA at 36 °C for 48 h in an anaerobic chamber (Bactron-I, SHELLAB, USA). The isolates were identified according to the manufacturer's guidelines. Briefly, a single colony was spotted on the target slide in a homogeneous smear, using a 1 μL loop. The bacteria were covered with 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution (bioMerieux, France). After drying at ambient temperature, the target slide was inserted into the VITEK MS machine (bioMérieux, France). Microbial identification was performed by comparing the generated spectra from the bacterial strains with the reference spectra present in the VITEK MS version 3.0 database. The data were categorized as follows: (I) accurate identification at the species level; (II) accurate identification at the genus level, including multiple *Prevotella* species that have low discrimination; (III) misidentification (minor error; different *Prevotella* species, major error; different genus) and (IV) no identification.

Each bacterium was spotted twice on the same target slide. If a good identification was not obtained, the spot was reanalyzed. For the misidentified or not-identified isolates, the test was repeated three times.

2.3. 16S rRNA gene sequencing

The VITEK MS identification was compared with the 16S rRNA gene sequencing identification. The bacterial DNA was extracted using the Quick-gDNA™ MiniPrep Kit (Zymo Research, USA) following the manufacturer's instructions. The 16S rRNA gene was amplified by using the universal 8UA, 907B, 774A and 1485B primers as described by Song et al. [16]. PCR products were sequenced using an ABI prims 3100 genetic analyzer (Applied Biosystems, Inc.) Sequences were analyzed using GenBank (www.ncbi.nlm.nih.gov) [17]. Identification at the species level was considered reliable if the sequence similarity with its closest relative was ≥99% [18].

3. Results

All isolates were Gram-negative, coccoid and short bacilli. After 3–5 days incubation on BBA supplemented with hemin and vitamin K, colonies vary from large to minute and were generally circular, entire, convex and smooth. *P. corporis, P. denticola, P. intermedia, P. melaninogenica, P. nigrescens* produce pigmented colonies varying from light brown to black. The colonies of *P. nigrescens* were also dry and brittle, it was hard to make a thin smear on the slide. In non-pigmented organisms *P. buccae* had the characteristic appearance of a mucoid colony.

Using 16S rRNA gene sequencing, all isolates were identified at the species level, representing 19 different *Prevotella* species (Table 1). The VITEK MS provided an accurate species identification for 422 (83.1%) of the isolates and an accurate genus identification for 459 (90.4%) of the isolates. The number of differences (n = 37) between correct species identification and genus identification is considered as a minor error, since species identification was incorrect and genus identification correct. A major error was encountered for 5 strains. Sequencing results showed that they were members of the genus *Prevotella*, but VITEK MS identified them to belong to the genera *Porphyromonas*, *Peptostreptococcus*, *Coronobacter* and for two strains the results were a mix of different genera (Table 2). Two of these strains belonged to *Prevotella* species (*P. bergensis* and *P. nanceiensis*) not represented in the VITEK MS database. Among the collection of all strains tested, 5 species were encountered, which were not represented in the VITEK MS database such as *P. bergensis*, *P. conceptionensis*, *P. corporis*, *P. histicola* and *P. nanceiensis.* These species represented 43 isolates (Table 1). Of these 43 isolates, 13 were correctly identified at the genus level, 28 were not identified and 2 strains were found to belong to a different genus (as *Porphyromonas asaccharolytica* and *Coronobacter sakazakii*) (as mentioned above) (Tables 1 and 2). The

Table 1
Identification of clinically relevant *Prevotella* isolates by the VITEK MS system.

Prevotella species	Species	Genus	Misidentified		NO ID (%)	Number of main spectra
identified by sequencing	Level (%)	Level (%)	Minor error (%)	Major error (%)		
P. baroniae (13)	11 (84.6)	12 (92.3)	1 (7.6)	0 (0.0)	1 (7.6)	1
***P. bergensis*[a] (13)**	0 (0.0)	2 (15.4)	2 (15.3)	1 (7.6)	10 (76)	0
P. bivia (118)	117 (99.1)	118 (100)	1 (0.8)	0 (0.0)	0 (0.0)	8
P. buccae (68)	63 (92.6)	64 (94.1)	1 (1.1)	1 (1.1)	3 (4.4)	9
P. buccalis (4)	4 (100)	4 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2
***P. conceptionensis*[a] (1)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0
***P. corporis*[a] (5)**	0 (0.0)	1 (20)	1 (20)	0 (0.0)	4 (80)	0
P. denticola (57)	52 (92.9)	56 (98.2)	4 (7)	0 (0.0)	1 (1.8)	6
P. disiens (31)	29 (93.5)	30 (96.7)	1 (3.2)	0 (0.0)	1 (3.2)	8
***P. histicola*[a] (11)**	0 (0.0)	8 (72.7)	8 (72.7)	0 (0.0)	3 (27.2)	0
P. intermedia (21)	17 (80.9)	19 (90.5)	2 (9.5)	1 (4.7)	1 (4.7)	7
P. melaninogenica (44)	41 (93.1)	42 (95.4)	1 (2.2)	0 (0.0)	2 (4.4)	5
***P. nanceiensis*[a] (13)**	0 (0.0)	2 (15.3)	2 (15.3)	1 (7.6)	10 (76.9)	0
P. nigrescens (62)	48 (77.4)	56 (90.3)	8 (90.3)	0 (0.0)	6 (9,6)	6
P. oralis (7)	7 (100)	7 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2
P. oris (16)	16 (100)	16 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3
P. salivae (11)	8 (72.7)	10 (90.9)	2 (18.2)	1 (9.1)	0 (0.0)	3
P. timonensis (9)	5 (55.5)	8 (88.8)	3 (33.3)	0 (0.0)	1 (11.1)	4
P. veroralis (4)	4 (100)	4 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3
Total: 508 (100%)	422 (83.1)	**459 (90.4)**	**37 (7.2)**	**5 (0.9)**	**44 (8.6)**	67

[a] Highlighted *Prevotella* species were missing from the VITEK MS V3.0 database.

Table 2
An overview of the major errors encountered when comparing the identification obtained by 16S rRNA gene sequencing and VITEK MS.

Identification by 16S rRNA gene sequencing	Identification by MALDI-TOF MS
P. bergensis	*Porphyromonas asaccharolytica*
P. buccae	*Peptostreptococcus anaerobius*
P. intermedia[a]	*Gemella sanguinis/Streptococcus alactolyticus/Pediococcus parvulus*
P. nanceiensis	*Coronobacter sakazakii*
P. salivae[a]	*Achromobacter denitrificans/S. salivarus/Bacteroides caccae*

[a] Low discrimination multiple genus with 33.3% confidence.

remaining 16 isolates which were not identified by the MALDI-TOF MS method during this study, belonged to such *Prevotella* species, which were represented in the VITEK MS database, even though VITEK MS was not able to identify them. Most of these strains (6/16) were *P. nigrescens*.

4. Discussion

In this multicenter study, we compared the utility and accuracy of VITEK MS v3.0 for the identification of *Prevotella* species, using16S rRNA gene sequencing as the reference method. Initial studies validating the use of MALDI-TOF MS for the identification of anaerobes reported that <70% of anaerobes could be identified [19–21]. However, more recent reports show that the current databases provide identification of >90% of anaerobes [9,22].

In earlier studies, only small numbers of *Prevotella* isolates representing different species were tested using MALDI-TOF MS, as part of the different collections of anaerobic bacteria [4–6,19,20,23]. So far two studies on identification of the *Prevotella* species have been conducted. The MALDI Biotyper system was used in both studies and the results were compared with 16S rRNA gene sequencing. In the first study by Wybo et al. in 2012 [14], a total of 102 clinical *Prevotella* isolates, representing 20 different species, were tested. Only 62.7% of the isolates were identified at the species level and 73.5% at the genus level. Expanding the commercial database with in-house reference spectra of 23 *Prevotella* reference strains and clinical isolates resulted in an increase of correct species identification and genus identification of 83.3% and 89.2%, respectively. In the second study by Gursoy et al. [15], the diagnostic accuracy of MALDI-TOF MS on a set of 123 oral *Prevotella* isolates was tested. Overall, 88.6% of isolates were correctly identified at the species level and 100% at the genus level. In the past five years, the accuracy of the MALDI Biotyper performance for identifying *Prevotella* species has improved considerably. The most likely explanation for the increased proportion of correct *Prevotella* identifications is the expanded coverage of the MALDI Biotyper database. The Biotyper database used by Wybo et al. [14] contained 20 species, whereas in the second study the database contained 30 *Prevotella* species [15].

A limited number of studies have been performed in which the VITEK MS was evaluated for the identification of anaerobic bacteria [10]. The first multi-center study evaluation the VITEK MS (v2.0) system was carried out by Garner et al. [24]. A total of 652 anaerobic clinical isolates were tested including 90 isolates representing five different *Prevotella* species. Broadly speaking, 91.1% of these strains were correctly identified at the species level. However, the evaluation of the results in more detail, the levels of correct identification at the species level for *P. intermedia* and *P. melaninogenica* were 81.3% and 54.5%, respectively. Compared to our study, more *Prevotella* strains were identified at the species level, probably due to the fact that only five commonly isolated species were represented. Our set of strains represented 19 different species, including species not represented in the database of the VITEK MS. As in our study, the VITEK MS failed to identify some strains of *P. intermedia* and *P. melaninogenica*, the percentage of correct identification at the species level were 80.9% and 93.1%, respectively. In the other study, Lee et al. [6] reported only genus level identification, misidentification or no identification for *P. buccalis*, *P. denticola* and

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P. melaninogenica. In an earlier study, Veloo at al [13]. detected beta-lactamase production in 100% of *P. melaninogenica* and 80% of *P. baroniae* species and also showed high rate resistance to tetracycline in these two species with 67% and 20%, respectively. These results point out that accurate identification of *Prevotella* isolates at the species level is crucial for initiation of appropriate empirical antibiotic therapy in case of infections.

In the study by Garner et al. [24] the results of no identification or misidentification as mixed genera for *P. melaninogenica* isolates were 9.1% or %18.2, respectively. The results for the additional of 12.5% of *P. intermedia* isolates were classified as no identification. In our study, the rate of no identification for *P. melaninogenica* and *P. intermedia* were 4.4% and 4.7%, respectively. Furthermore, we noticed that 6 (9.6%) strains of *P. nigrescens,* and some *P. baroniae*, *P. buccae*, *P. denticola*, *P. timonensis,* which would have been represented in IVD VITEK MS database, were also unidentified. One reason for a no identification result may be the small number of main spectra (MSP) in the database. The Reference Library SAP 2436056 IVD VITEK MS contains a total of 67 *Prevotella* MSPs of 14 *Prevotella* species. However, two species (one of those *P. baroniae*) are represented by a single MSP, and three species represented by two MSPs for each, which can affect the correct identification. This indicates that the VITEK MS database needs optimization for these species. Another reason might be the pigmented nature of the organisms. It has been reported that certain microorganisms, which have pigments like melanin and other photoactive compounds may hinder the quality of the spectra obtained [25]. 10 of 16 of our unidentified isolates, which have MSP in the VITEK MS database, were pigmented *Prevotella* species. Our personal experience suggests that identifying pigmented bacteria is more troublesome and frequently the procedure has to be repeated. However, we have no investigation of the effect of bacterial pigment on acquired MALDI–TOF mass spectra, to support this hypothesis.

Five strains in our study were identified with a major error (wrong genus); *P. bergensis*, *P. buccae*, *P. intermedia*, *P. nanceiensis* and *P. salivae*. Similar findings have been reported by Lee et al. [6] who observed a strain of *P. buccalis* being misidentified as *Pseudoflavonifractor capillosus*, using VITEK MS. The antimicrobial susceptibility patterns may markedly be different between genera even between species. Since the resistance to penicillin has been reported in about half of *Prevotella* spp., in our case, use of penicillin on *Prevotella* isolates misidentified as *P. anaerobius, Gemella* or *Pediococcus,* which mostly susceptible to penicillin, may cause failure of empirical treatment [12,26,27]. Also, for these species, optimization of the VITEK MS database is recommended, in order to prevent misidentification, thereby enabling selection of the most appropriate antibacterial therapy and reducing morbidity and mortality.

This is the first study assessing the applicability of the VITEK MS for the identification of human clinical *Prevotella* that uses an extensive set of isolates covering 19 different species. In general, the VITEK MS system performed well in the identification of clinically important, frequently found *Prevotella* strains. However, the VITEK MS database needs to be expanded and optimized by adding reference spectra of species not yet represented and of species underrepresented in the database. Accurate and easy identification of clinical isolates increases interest in studies dealing with anaerobic organisms, and improves our knowledge of clinical relevance, epidemiology and the pathogenicity of *Prevotella* species.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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